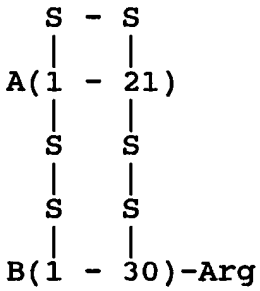
21. (Amended) A method for the preparation of a mono-Arg-insulin compound of [the] formula II




(II)

in which A(1-21) and B(1-30) denote the A and B chains of human insulin and the -S-S- bridges are positioned as in insulin, [using the compound of the formula I] which comprises:

(a) expressing as part of a fusion protein in a bacterium a DNA molecule encoding a mini-proinsulin compound of the formula [I in a bacterium]:

B(1-30)-Arg-A(1-21),

in which B(1-30) and A(1-21) denote the B and A chains of human insulin; [and]

(b) liberating said mini-proinsulin compound from said fusion protein to obtain said mini-proinsulin in a native conformation; and

[(b)] (c) incubating [the expressed] said mini-proinsulin compound [of the formula I resulting from step (a)] with trypsin [under slightly acidic conditions] at a pH of about 6.8 [where phenol and other similar aromatics are not present] under conditions where no crystals are formed.

LAW OFFICES
FINNEGAN, HENDERSON
FARABOW, GARRETT
& DUNNER
1300 I STREET, N.W.
WASHINGTON, DC 20005
1-202-408-4000

22. (Amended) A method for the preparation of insulin [using the compound of the formula I] which comprises:

(a) expressing as part of a fusion protein in a bacterium a DNA molecule encoding the compound of the formula [I in a bacterium]:

B(1-30)-Arg-A(1-21),

in which B(1-30) and A(1-21) denote the B and A chains of human insulin;

(b) liberating said mini-proinsulin compound from said fusion protein to obtain said mini-proinsulin in a native conformation;

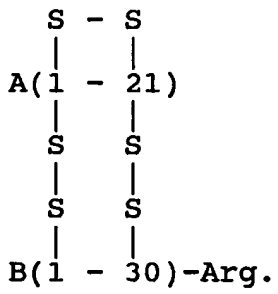
[(b)] (c) incubating [the expressed] said mini-proinsulin compound [of the formula I resulting from step (a)] with trypsin [under slightly acidic conditions] at a pH of about 6.8 [where phenol and other similar aromatics are not present] under conditions where no crystals are formed to produce a mono-Arg-insulin; and

[(c)] (d) cleaving the resulting mono-Arg-insulin [compound of the formula II] with carboxypeptidase B.

23. (Amended) A method as claimed in claim [7] 22, wherein steps [(b) and] (c) and (d) are carried out in one vessel without

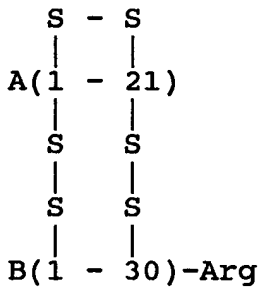
LAW OFFICES
FINNEGAN, HENDERSON
FARABOW, GARRETT
& DUNNER
1300 I STREET, N.W.
WASHINGTON, DC 20005
1-202-408-4000

having to isolate as an intermediate [compound] mono-Arg-insulin of [the] formula II




(II)

25. (Amended) A method for the preparation of a mono-Arg-insulin compound of [the] formula II




(II)

in which A(1-21) and B(1-30) denote the A and B chains of human insulin and the -S-S- bridges are positioned as in insulin, which comprises:

(a) expressing in a bacterium a DNA molecule encoding [the] a fusion protein [of claim 9 in a bacterium] which comprises

B(1-30)-Arg-A(1-21)

bonded via a bridging member,

- Met - Ile - Glu - Gly -Arg -,

to a peptide which stabilizes the fusion protein;

(b) liberating said mini-proinsulin compound from said fusion protein by cleaving the expressed fusion protein resulting from step (a) with cyanogen bromide, to obtain said

LAW OFFICES
FINNEGAN, HENDERSON
FARABOW, GARRETT
& DUNNER
1300 I STREET, N.W.
WASHINGTON, DC 20005
1-202-408-4000

mini-proinsulin in a native conformation [thereby producing mini-proinsulin]; and

(c) incubating the mini-proinsulin of step (b) with trypsin [under slightly acidic conditions] at a pH of about 6.8 [where phenol and other similar aromatics are not present] under conditions where no crystals are formed.

26. (Amended) A method for the preparation of insulin which comprises:

(a) expressing in a bacterium a DNA molecule encoding [the] a fusion protein [of claim 9 in a bacterium] which comprises

B(1-30)-Arg-A(1-21)

bonded via a bridging member,

- Met - Ile - Glu - Gly -Arg -,

to a peptide which stabilizes the fusion protein;

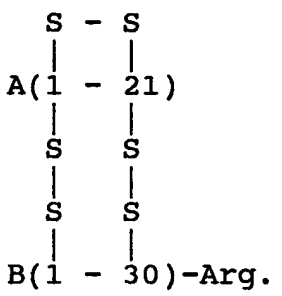
(b) liberating said mini-proinsulin compound from said fusion protein by cleaving the expressed fusion protein resulting from step (a) with cyanogen bromide, to obtain said mini-proinsulin in a native conformation [thereby producing mini-proinsulin];

(c) incubating the mini-proinsulin of step (b) with trypsin [under slightly acidic conditions] at a pH of about 6.8 [where phenol and other similar aromatics are not present] under conditions where no crystals are formed to produce a mono-Arg-insulin; and

(d) cleaving the resulting [compound of the formula II] mono-Arg-insulin with carboxypeptidase B.

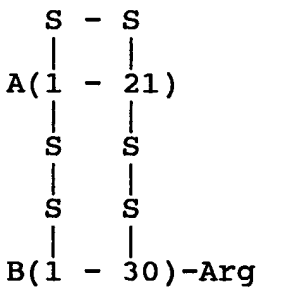
LAW OFFICES
FINNEGAN, HENDERSON
FARABOW, GARRETT
& DUNNER
1300 I STREET, N.W.
WASHINGTON, DC 20005
1-202-408-4000

27. (Amended) A method as claimed in claim [11] 26, wherein steps (c) and (d) are carried out in one vessel without having to isolate as an intermediate [compound] mono-Arg-insulin of the formula II




(II)

29. (Amended) A mono-Arg-insulin compound of [the] formula II




(II)

in which A(1-21) and B(1-30) denote the A and B chains of human insulin and the -S-S- bridges are positioned as in insulin, which is formed by the process which comprises:

(a) expressing as part of a fusion protein in a bacterium a DNA molecule encoding the compound of the formula [I in a bacterium]:

B(1-30)-Arg-A(1-21)

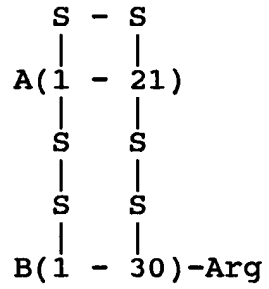
in which B(1-30) and A(1-21) denote the B and A chains of human insulin;

LAW OFFICES
FINNEGAN, HENDERSON
FARABOW, GARRETT
& DUNNER
1300 I STREET, N.W.
WASHINGTON, DC 20005
1-202-408-4000

(b) liberating said mini-proinsulin compound from said fusion protein to obtain said mini-proinsulin in a native conformation; and

[(b)] (c) incubating [the expressed] said mini-proinsulin compound [of the formula I resulting from step (a)] with trypsin [under slightly acidic conditions] at a pH of about 6.8 [where phenol and other similar aromatics are not present] under conditions where no crystals are formed.

30. (Amended) A mono-Arg-insulin compound of [the] formula II




(II)

in which A(1-21) and B(1-30) denote the A and B chains of human insulin and the -S-S- bridges are positioned as in insulin, which is formed by the process which comprises:

(a) expressing in a bacterium a DNA molecule encoding [the] a fusion protein [of claim 9 in a bacterium] which comprises

B(1-30)-Arg-A(1-21)

bonded via a bridging member,

- Met - Ile - Glu - Gly -Arg -,

to a peptide which stabilizes the fusion protein;

(b) liberating said mini-proinsulin compound from said fusion protein by cleaving the expressed fusion protein resulting from step (a) with cyanogen bromide, to obtain said

LAW OFFICES
FINNEGAN, HENDERSON
FARABOW, GARRETT
& DUNNER
1300 I STREET, N.W.
WASHINGTON, DC 20005
1-202-408-4000